Figure S1. PhCM1 and PhCM2 CDS alignment using the Align X program in Vector NTI advance 10.3.0 software package (Invitrogen; Carlsbad, CA, USA). PhCM1 and PhCM2 share 46.1% identity at the nucleotide level.

Figure S2. qRT-PCR transcript accumulation analysis of PhCM1 and PhCM2 in petunia. Spatial analysis used root, stem, stigma, anther, leaf, petal tube, petal limb, and sepal tissues of MD harvested at 16:00 h (a). The spatial experiment consisted of one biological replicate used for sqRT-PCR and one separate biological replicate with two technical replicates per biological replicate. Floral developmental analysis used MD flowers from 11 sequential stages at 16:00 h (b). The developmental analysis consisted of two biological replicates separate from the biological replicates used for the sqRT-PCR with three technical replicates. Ethylene treatment (2 μl L-1) analysis used excised MD and 44568 whole flowers treated for 0, 1, 2, 4, and 8 h (c). The ethylene treated series consisted of one biological replicate used in the sqRT-PCR with two technical replicates per biological replicate. PhFBP1 and Ph18S were used as references throughout these experiments.

Figure S3. Schematic representation and nucleotide comparison of RNAi region used for the production of petunia PhCM1 RNAi transgenic lines. 213 bases at the 3′ end of the coding sequence of PhCM1 were chosen for the RNAi construct. This region shared 58.2% identity with the corresponding nucleotide region from PhCM2.

Figure S4. sqRT-PCR transcript accumulation analysis in floral tissues of three independent T1 ir-PhCM1 lines. MD, 2-4, 24-9, 33-9 were used with primers specific for floral volatile benzenoid/phenylpropanoid, shikimate, and phenylpropanoid transcripts. The number of cycles used for amplification of each transcript is shown on the right. Ph18S was used as a loading control in all cases.

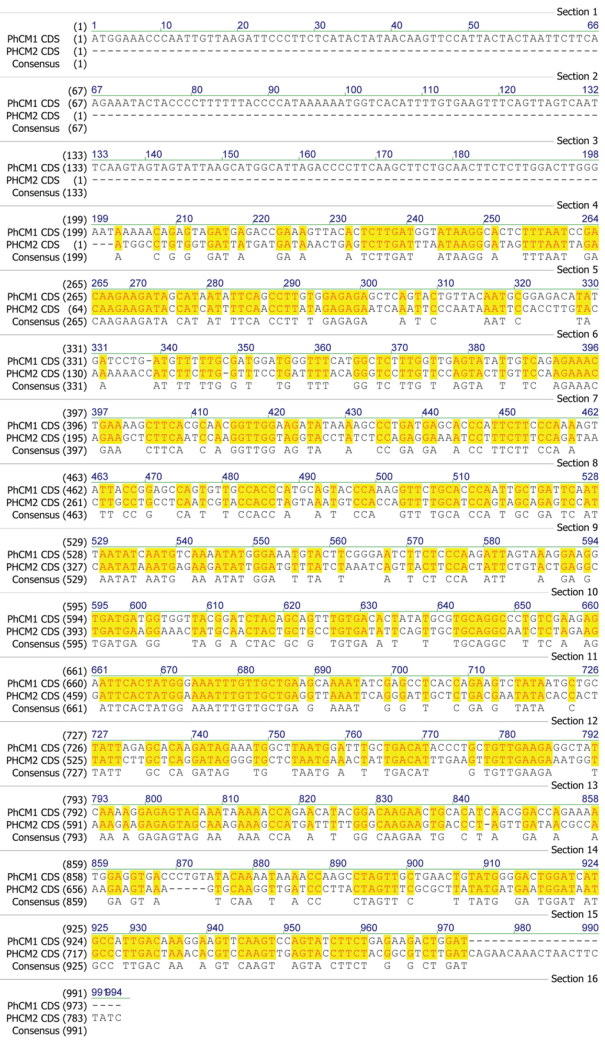
Figure S5. sqRT-PCR transcript accumulation analysis in floral tissues of two independent, homozygous T2 ir-PhCM1 lines. Individuals and biological replicates from MD, 24-9, 33-8 were used with primers specific for PhCM1. The number of cycles used for amplification of each transcript is shown on the right. Ph18S was used as a loading control.

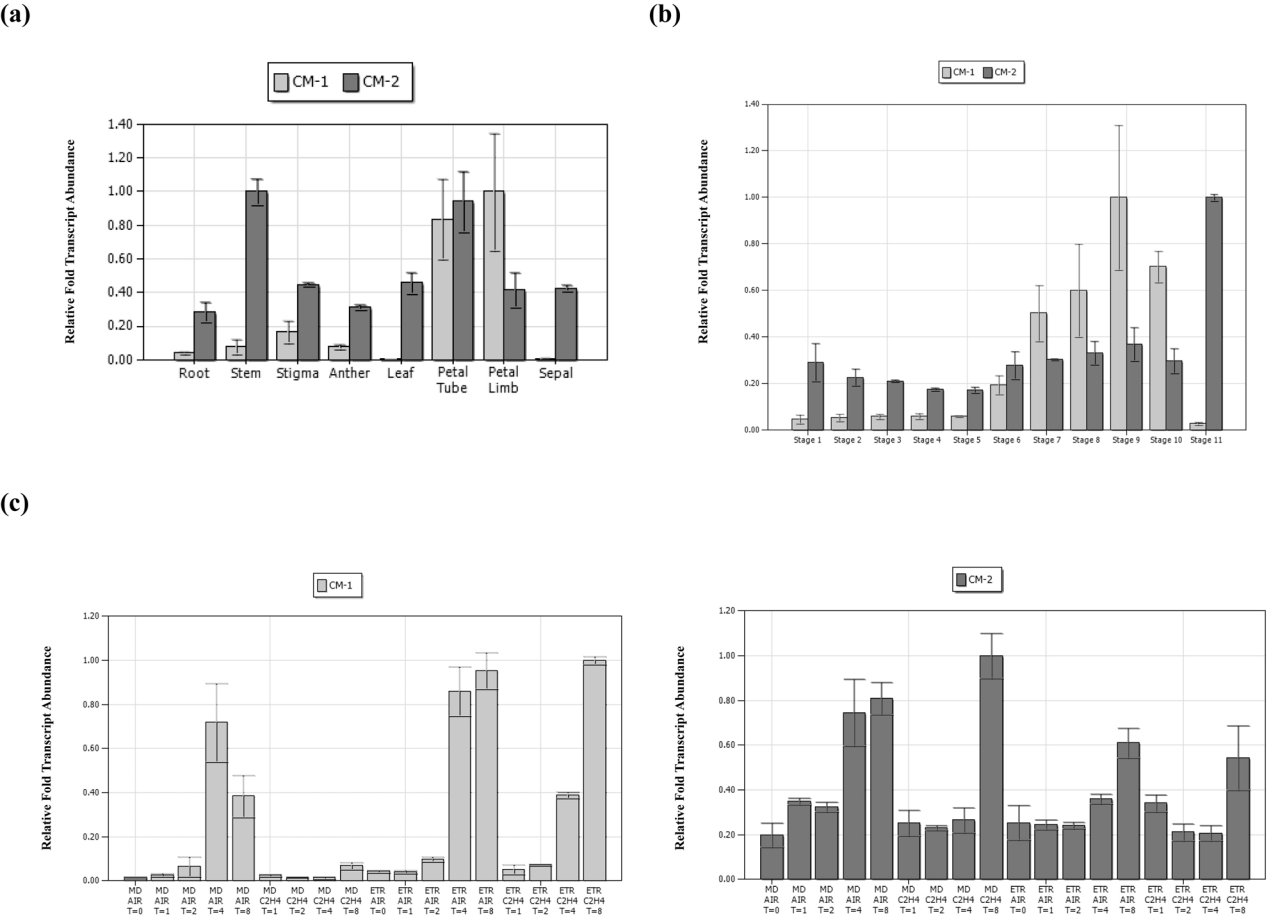
Figure S6. Physiological comparison between MD and representative independent T2 ir-PhCM1 homozygous lines 24-9 and 33-8 in 9 week old petunia seedlings (mean ± SE; n = 5).

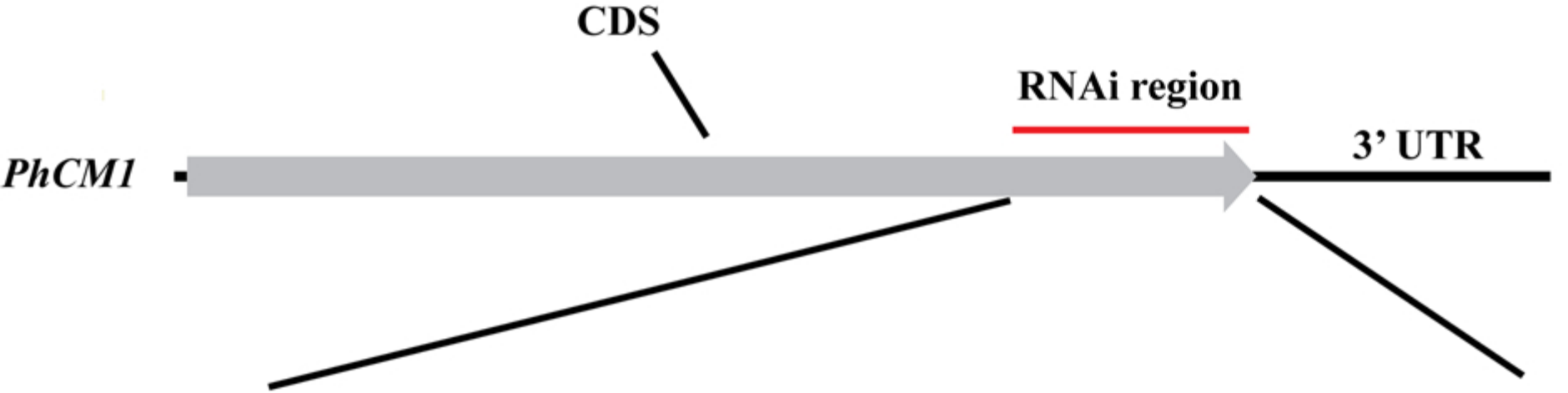
Figure S7. Stem cross-sections (between 7–8 node from apical meristem) from 9 week old petunias stained with Phloroglucinol. Shown are MD and representative individuals from two independent ir-PhCM1 homozygous T2 lines, 24-9 and 33-8. Pictures are from light microscopy at 4 × on a Leica MZ 16F and are representative of three biological replicates.

Table S1. Functional complementation of CM-deficient E. coli KA12/pKIMP-UAUC. M9c minimal media was used for all experiments and supplemented with 20 μg ml−1 of L-phenylalanine and L-tyrosine where stated. Antibiotics used were chloramphenicol [Ch] (30 μg ml−1) for selection of the pKIMP plasmid and carbenicillin [Ca] (100 μg ml−1) for selection of the pET-32 plasmid. KA12/pKIMP-UAUC is not a λDE3 lysogenic E. coli, so bacteriophage CE6 (Novagen, cat# 69390) infection was used to induce transcription from the pET-32 T7 promoter where stated and no CE6 administered (NA) where stated. Transformants were incubated at 37°C for two days, and growth was scored as a plus (+) or minus (−). A sample of positive colonies was picked and colony PCR was performed for confirmation of pET-32-CM1 or pET-32-CM2 plasmids.

Table S2. Gene specific primers used for the transcript accumulation analyses throughout this study.


Section 1
PhCM1 CDS
PhCM2 CDS
Consensus
Section 2
PhCM1 CDS
PhCM2 CDS
Consensus
Section 3
PhCM1 CDS
PhCM2 CDS
Consensus
Section 4
PhCM1 CDS
PhCM2 CDS
Consensus
Section 5
PhCM1 CDS
PhCM2 CDS
Consensus
Section 6
PhCM1 CDS
PhCM2 CDS
Consensus
Section 7
PhCM1 CDS
PhCM2 CDS
Consensus
Section 8
PhCM1 CDS
PhCM2 CDS
Consensus
Section 9
PhCM1 CDS
PhCM2 CDS
Consensus
Section 10
PhCM1 CDS
PhCM2 CDS
Consensus
Section 11
PhCM1 CDS
PhCM2 CDS
Consensus
Section 12
PhCM1 CDS
PhCM2 CDS
Consensus
Section 13
PhCM1 CDS
PhCM2 CDS
Consensus
Section 14
PhCM1 CDS
PhCM2 CDS
Consensus
Section 15
PhCM1 CDS
PhCM2 CDS
Consensus
PhCM1 CDS
PhCM2 CDS
Consensus


(a)
CM-1
CM-2
Relative Fold Transcript Abundance
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
Root
Stem
Stigma
Anther
Leaf
Petal Tube
Petal Limb
Sepal
(b)
CM-1
CM-2
Relative Fold Transcript Abundance
Stage 1
Stage 2
Stage 3
Stage 4
Stage 5
Stage 6
Stage 7
Stage 8
Stage 9
Stage 10
Stage 11
(c)
CM-1
Relative Fold Transcript Abundance
MD AIR T=0
MD AIR T=1
MD AIR T=2
MD AIR T=4
MD AIR T=8
MD C2H4 T=1
MD C2H4 T=2
MD C2H4 T=4
MD C2H4 T=8
ETR AIR T=0
ETR AIR T=1
ETR AIR T=2
ETR AIR T=4
ETR AIR T=8
ETR C2H4 T=1
ETR C2H4 T=2
ETR C2H4 T=4
ETR C2H4 T=8
CM-2
Relative Fold Transcript Abundance
MD AIR T=0
MD AIR T=1
MD AIR T=2
MD AIR T=4
MD AIR T=8
MD C2H4 T=1
MD C2H4 T=2
MD C2H4 T=4
MD C2H4 T=8
ETR AIR T=0
ETR AIR T=1
ETR AIR T=2
ETR AIR T=4
ETR AIR T=8
ETR C2H4 T=1
ETR C2H4 T=2
ETR C2H4 T=4
ETR C2H4 T=8




```
PhCM1  (760) TTGCTGACATACCCTGCTGTTGAAGAGGCTATCAAAAGGAGAGTAG AAAT
PhCM2  (562) CTATTGACATTTGAAGTTGTTGAAGAAATGGTAAAGAAGAGAGTAGCAAA
                T  TGACAT       G TGTTGAAGA       T AA A GAGAGTAG AA

PhCM1  (810) AAAAACCAGAACATACGGACAAGAACTGCACATCAACGGACCAGAAAATG
PhCM2  (612) GAAAGCCATGATTTTTGGGCAAGAAGTG–ACCCTAGTTGATAACGCCAAA
                AAA CCA   A   T   GG CAAGAA TG AC   A    GA  A     A

PhCM1  (860) GAGGTGACCCTGTATACAAAATAAAACCAAGCCTAGTTGCTGAACTGTAT
PhCM2  (662) GAAGTAA–––––AGTGCAAGGTTGATCCCTTACTAGTTTCGCGCTTATAT
                GA GT A         T CAA  T   A CC      CTAGTT C       T TAT

PhCM1  (910) GGGGACTGGATCATGCCATTGACAAAGGAAGTTCAAGTCCAGTATCTTCT
PhCM2  (712) GATGAATGGATAATGCCCTTGACTAAACACGTCCAAGTTGAGTACCTTCT
                G   GA TGGAT ATGCC TTGAC AA   A GT CAAGT   AGTA CTTCT

PhCM1  (960) GAGAAGACTGGAT
PhCM2  (762) ACGGCGTCTTGAT
                G  G CT GAT
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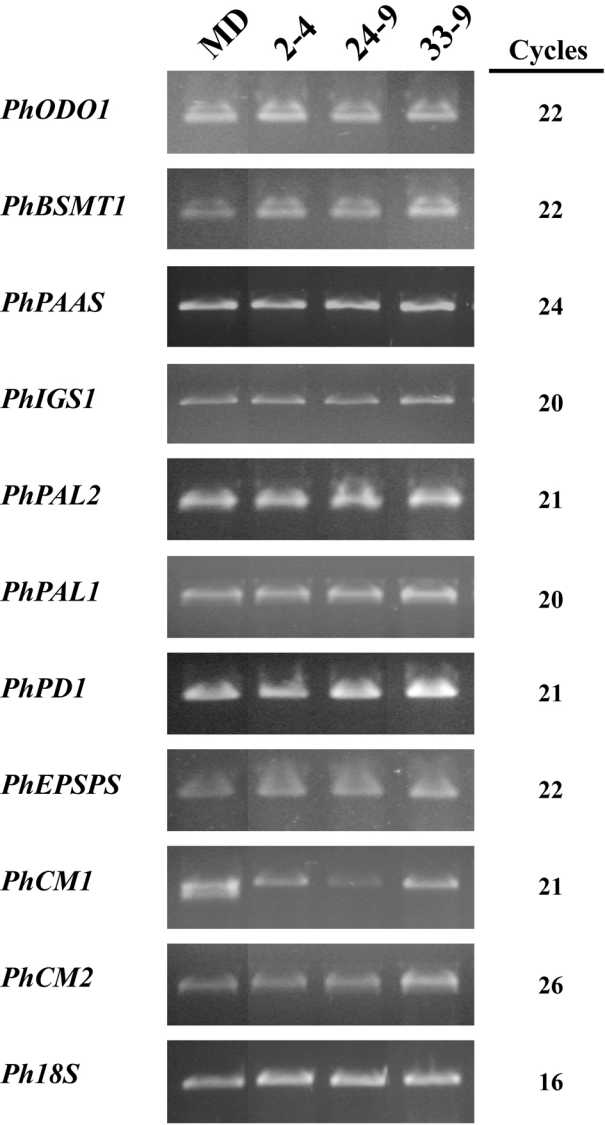
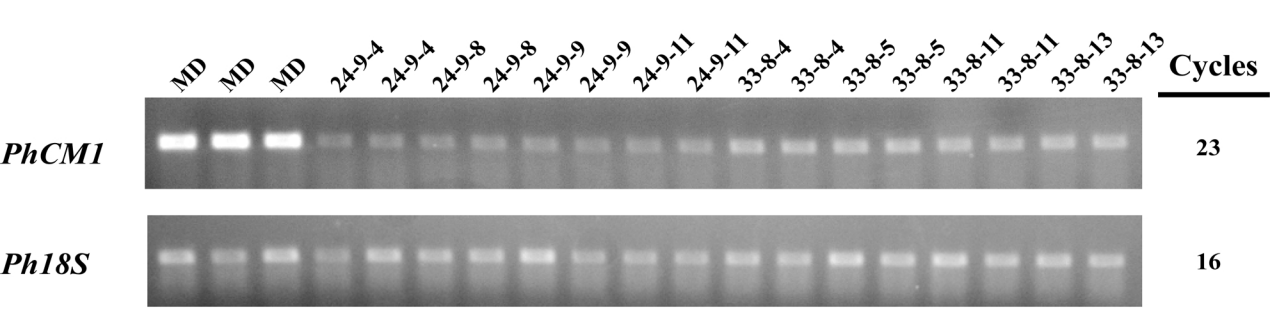

MD
2-4
24-9
33-9
Cycles
PhODO1
22
PhBSMT1
22
PhPAAS
24
PhIGS1
20
PhPAL2
21
PhPAL1
20
PhPD1
21
PhEPSPS
22
PhCM1
21
PhCM2
26
Ph18S
16


MD
MD
MD
24-9-4
24-9-4
24-9-8
24-9-8
24-9-9
24-9-9
24-9-11
24-9-11
33-8-4
33-8-4
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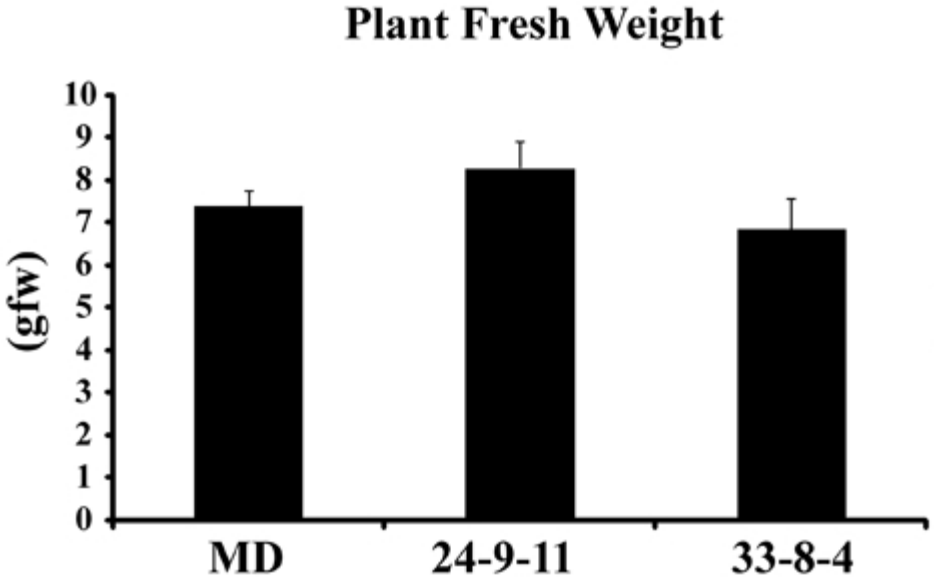
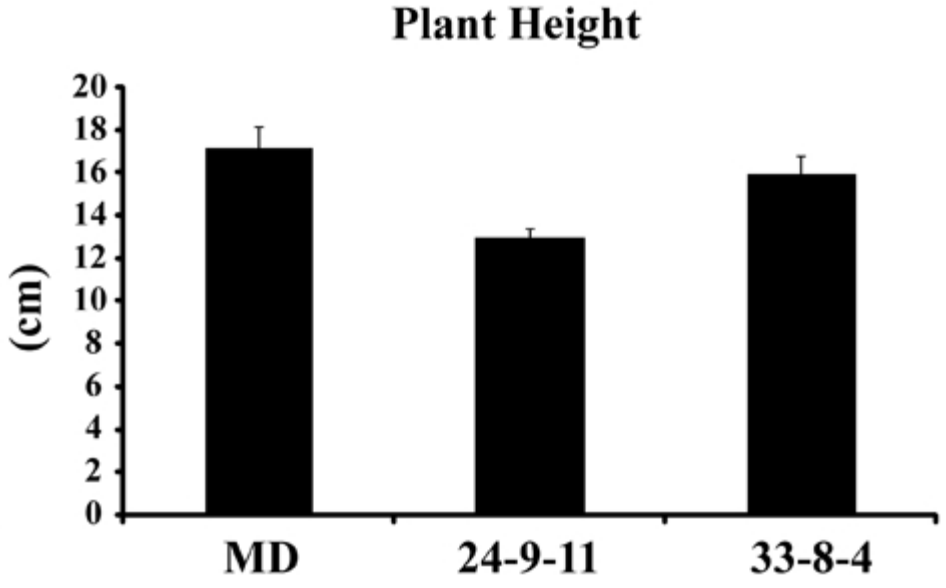
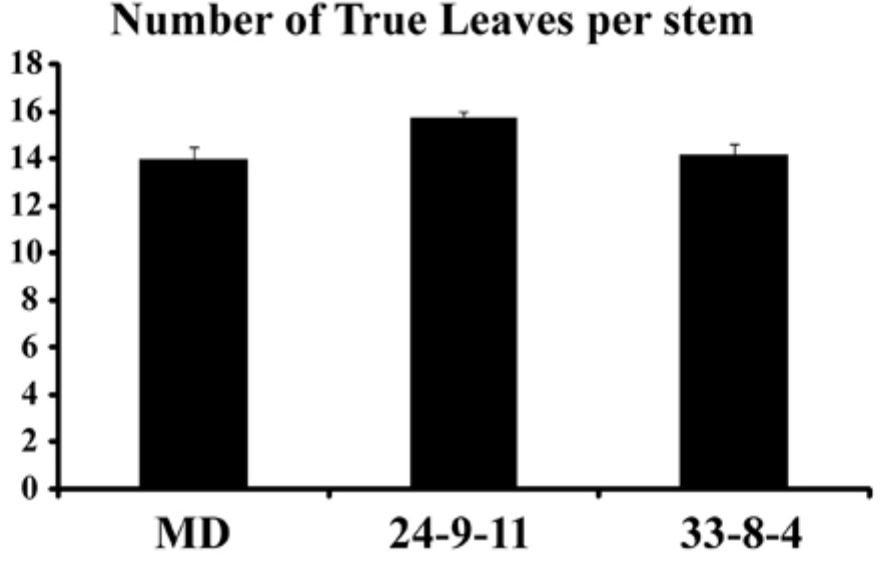
Cycles
PhCM1
23
Ph18S
16


Plant Fresh Weight
(gfw)
10
9
8
7
6
5
4
3
2
1
0
MD
24-9-11
33-8-4

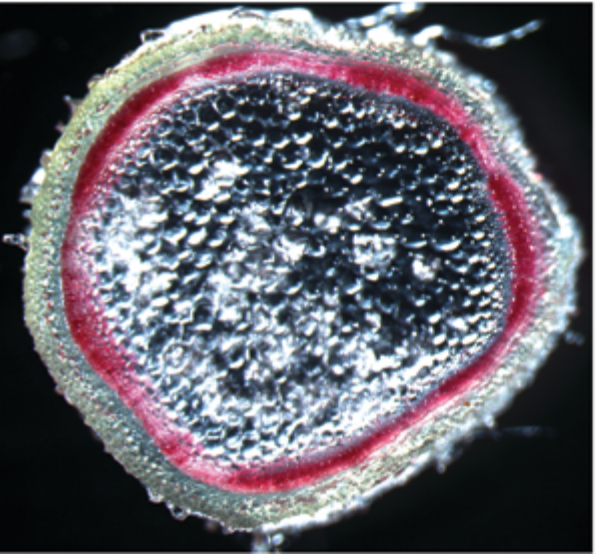

Plant Height
(cm)
20
18
16
14
12
10
8
6
4
2
0
MD
24-9-11
33-8-4

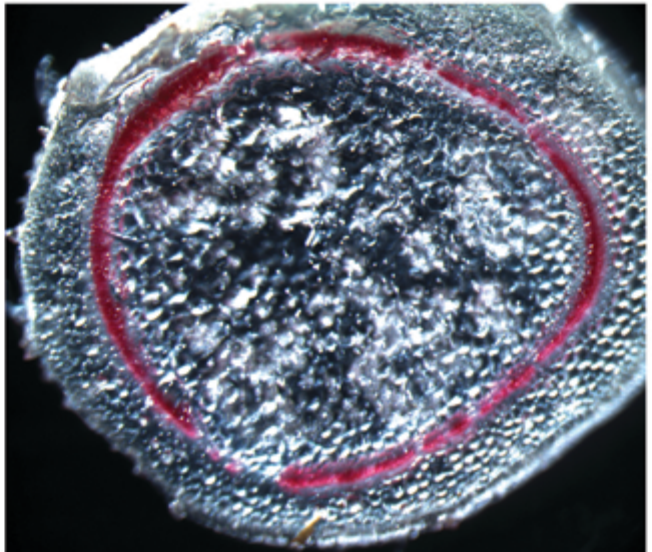

Number of True Leaves per stem
18
16
14
12
10
8
6
4
2
0
MD
24-9-11
33-8-4

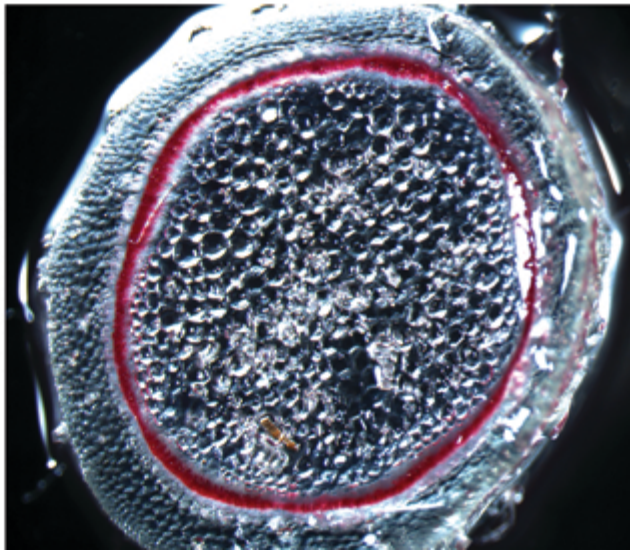
MD

24-9-11

33-8-4







Bacteria/Plasmid	Media	Antibiotic	Treatment	Growth
KA12/pKIMP-UAUC	M9c/Phenylalanine, Tyrosine	Ch	NA	+
KA12/pKIMP-UAUC	M9c	Ch	NA	-
KA12/pKIMP-UAUC	M9c/Phenylalanine, Tyrosine	Ch	CE6	+
KA12/pKIMP-UAUC	M9c	Ch	CE6	-
KA12/pKIMP-UAUC	M9c/Phenylalanine, Tyrosine	Ch/Ca	NA	-
KA12/pKIMP-UAUC	M9c	Ch/Ca	NA	-
KA12/pKIMP-UAUC	M9c/Phenylalanine, Tyrosine	Ch/Ca	CE6	-
KA12/pKIMP-UAUC	M9c	Ch/Ca	CE6	-
KA12/pKIMP-UAUC/pET-32-CM1	M9c/Phenylalanine, Tyrosine	Ch/Ca	NA	+
KA12/pKIMP-UAUC/pET-32-CM1	M9c	Ch/Ca	NA	-
KA12/pKIMP-UAUC/pET-32-CM1	M9c/Phenylalanine, Tyrosine	Ch/Ca	CE6	+
KA12/pKIMP-UAUC/pET-32-CM1	M9c	Ch/Ca	CE6	+
KA12/pKIMP-UAUC/pET-32-CM2	M9c/Phenylalanine, Tyrosine	Ch/Ca	NA	+
KA12/pKIMP-UAUC/pET-32-CM2	M9c	Ch/Ca	NA	-
KA12/pKIMP-UAUC/pET-32-CM2	M9c/Phenylalanine, Tyrosine	Ch/Ca	CE6	+
KA12/pKIMP-UAUC/pET-32-CM2	M9c	Ch/Ca	CE6	+

Reference Number	Target	Direction	Primer Sequence (5'→3')
sqRT-PCR			
AJ236020	*Ph18S*	Forward	TTAGCAGGCTGAGGTCTCGT
		Reverse	AGCGGATGTTGCTTTTAGGA
AY233465	*PhBSMT1*	Forward	AGAAGGAAGGATCATTCACCA
		Reverse	TATTCGGGTTTTTCGACCAC
EU751616	*PhCM1*	Forward	GCCATTGACAAAGGAAGTTCA
		Reverse	TGTTCCAAACTTGAAAATTACATCA
EU751617	*PhCM2*	Forward	CCATCTTCTTGGTTTCCTGA
		Reverse	TCACAGGCAGCAGTAGTTGC
M21084	*PhEPSPS*	Forward	CGATGATCACAGGATGGCCATGG
		Reverse	CCCATTGTGGTTCTGATACCAG
DQ372813	*PhIGS1*	Forward	GCCTATGTCATGCCATTGAA
		Reverse	TGCTTTAATTGTGTAGGCTGC
AY705977	*PhODO1*	Forward	TTCAATTGGCTTTCGGGTTA
		Reverse	AGGCACCTTGGACTCTTCG
DQ243784	*PhPAAS*	Forward	TCCTTGTAGTTCTAGTACTGCTGGAA
		Reverse	TCAACAGCAGTTGTTGAAGTAGTTC
AY705976	*PhPAL1*	Forward	GCTATGAATGAAGGAAAGTTGG
		Reverse	CACAATCTTTCATACAAACCC
CO805160	*PhPAL2*	Forward	CCGAGCTGTTGACAGGAGAAGG
		Reverse	TAACCAGACTACTAAAGTTCAGC
SGN-U207570	*PhPD1*	Forward	ACATCGCAGCCAATAACCTT
		Reverse	CCCACATTTGCATCATCAAC
qRT-PCR			
AJ236020	*Ph18S*	Forward	TGCAACAAACCCCGACTTCT
		Reverse	AGCCCGCGTCAACCTTTTAT
EU751616	*PhCM1*	Forward	CCCTGATGAGCACCCATTC
		Reverse	ACTGCATGGGTGGCAACAC
EU751617	*PhCM2*	Forward	AACTTGCCTGCCTCAATCGT
		Reverse	GGATGCAAAACTGGTGGACAT
M91190	PhFBP1	Forward	TGCGCCAACTTGAGATAGCA
		Reverse	TGCTGAAACACTTCGCCAATT